The Journal of Animal & Plant Sciences, 31(4): 2021, Page: 1070-1077
ISSN (print): 1018-7081; ISSN (online): 2309-8694

DETERMINATION OF LETHAL AND FEEDING DETERRENT ACTIVITIES OF SAPONIN FROM *PHALERIA MACROCARPA* AGAINST *POMACEA MACULATA*

A. R. Roonjho, R. Muhamad* and D. Omar

Department of Plant Protection, Faculty of Agriculture Universiti Putra Malaysia
Corresponding Author's email: rita@upm.edu.my

ABSTRACT

Apple snail is one of the major pest of rice crop and saponin proved to be the most promising bioactive compound to control it. This study was carried out to quantify saponin from God's Crown, *Phaleria macrocarpa* and to evaluate its efficacy against the biological activities of black apple snail, *Pomacea maculata*. Fruits, leaves and stem-barks of *P. macrocarpa* were quantified for saponin using HPLC. The toxicity of leaf and fruit crude extracts was evaluated through mortality and feeding deterrent bioassays using complete randomized design and data were analyzed by ANOVA for LSD test. The highest saponin contents 24.67 ppm was detected in fruits followed by 22.67 ppm in leaves and 5.94 ppm in stem-bark. Bioassays showed the highest mortality percentage (44%) after 24 hours exposure at the concentration of 1000 ppm of a leaf extract followed by 36% and 28% @ 750 and 1000 ppm of leaf and fruit extracts, respectively. After the exposure of 48 hours, mortality percentage increased to 100% @ 1000 and 750 ppm of both crude extracts while the mortality percentage recorded at the concentration of 500 ppm of leaves and fruits were 56% and 52% respectively. Mortality percentage at the concentration of 500 ppm was increased to 80% and 68% in leaf and fruit extracts after exposure of 72 hours, respectively. In terms of feeding deterrent, 1000, 750 and 500 ppm concentration of both crude extracts were not significantly different from the positive control niclosamide ($p>0.05$). The results obtained from the study revealed that saponin extracted from fruits and leaves of *P. macrocarpa* has a potential to control black apple snails.

Key words: *Phaleria macrocarpa*, *Pomacea maculata,* HPLC, botanical molluscicides, saponins

https://doi.org/10.36899/JAPS.2021.4.0304 *Published online December 18, 2020*

INTRODUCTION

Pomacea spp., generally known as apple snail, belong to Family Ampullariidae from the phylum Mollusca. Apple snail is a well-known pest of rice crops in many Asian countries (Mokhtar, 2016). Historically, the pest is a South American native and introduced in Asia around 1980s as a food resource, but later it becomes a grievous pest of rice crop in many rice growing East Asian countries (Cowie, 2005; Naylor, 1996). Currently, apple snail has extended from Asia to USA, Australia and latest being in Spain, which makes it the first recorded infestation in Europe (Cowie, 2005, 1998; Eldredge, 1994; Rawlings *et al*., 2007). Apple snail has been acknowledged as one of the 100 invasive alien species found in the world (Lowe *et al*., 2000). *Pomacea maculata* (Black apple snail) and *Pomacea canaliculata* (Golden apple snail) mostly infest rice fields throughout the world (Hayes *et al*., 2012; Yahaya *et al*., 2007). Both species can be differentiated through the colour and suture structure of the shells, whereby, *P. canaliculata* is yellowish to golden with short and deeply channelled suture whereas, *P. maculata* is black with longer suture (Hayes *et al*., 2012). Black apple snails are more abundantly distributed in Malaysia than golden apple snails (Arfan *et al*., 2014). Mostly synthetic molluscicides are being used against the snails but unfortunately; these molluscicides are also well known for their adverse effects on human and environment (Mokhtar, 2016). Among the botanicals, saponin is the most promising and widely studied bioactive compound against apple snails (Mokhtar, 2016; Hostettmann *et al*., 1982; San Martin, 2007). Numerous studies have revealed that the haemolytic properties of saponin affect biological activities, thus, making it highly toxic to most cold-blooded pests. In snails, it causes apoptosis, which leads to uncontrolled cell death (De Geyter *et al*., 2007; Sparg *et al*., 2004). *Phaleria macrocarpa* also known as mahkota dewa or God's crown, it is a medicinal plant indigenous to Indonesia and Malaysia. The leaves, stem barks and fruits of *P. macrocarpa* are widely known for its medicinal purpose and have been used since years as traditional medicines in Indonesia and Malaysia to treat breast cancer, bone cancer, heart and liver diseases, tumours and diabetes (Kim *et al*., 2010; Hending, 2009). The fruits of *P. macrocarpa* are good source of saponin (Altaf *et al*., 2013; Gotama *et al*., 1999). The stem-barks and leaves of *P. macrocarpa* have also been reported to have saponin bioactive compound (Altaf *et al*., 2013; Andrean *et al*., 2014; Gotama *et al*., 1999; Tjandrawinata *et al*., 2011). Thus, this study was conducted to quantify saponin bioactive compound in *P. macrocarpa* and its efficacy on the biological activities of *P. maculata*.

MATERIALS AND METHODS

Collection and preparation of plant materials: Leaves, fruits and stem-barks of *P. macrocarpa* were collected in the month of June 2016 from Taman Pertanian Universiti, Universiti Putra Malaysia. The collected materials were properly washed using tap water, and then followed by distilled water. Plant materials were then dried in oven at 45 °C for one week. The dried plant materials were then pulverized using a grinder, and the powdered material was passed through a 0.7 mm sieve (attached with grinder) to obtain a finer dust.

Extraction: The extraction of saponin was performed through the maceration extraction method described by Takeuchi *et al*. (2009) with slight modifications. Methanol was used as a solvent to isolate the bioactive compound from plant samples (Mustarichie *et al*., 2012). A hundred grams of each sample was placed into a 1000 ml beaker and mixed with 700 mL of the solvent. The mixture was shaken for four days using an orbital shaker (Protech) and left to stand for the next 24 hours. Subsequently, the mixtures were filtered twice, once through a fine cloth and again through Whatman No.1 filter paper and finally the filtrates were ensured to be solvent free using the Rotavapour R-215 that was connected to a heating bath B-149 at 40 °C and vacuum pump V-700 (BUCHI, United Kingdom) at 100 RPM. The crude extracts in the flask were transferred into glass vials and stored in a refrigerator at -4 ºC until their next use.

Quantification of saponin bioactive compound: The quantification of saponin bioactive compound was performed on Agilent 1100 series HPLC system with DAD Diode Array Detector (Agilent Technologies, USA). The method used was modified from Guo *et al*. (2011) and standard saponin of analytical grade from Sigma-Aldrich, USA was used as external reference of saponin. The Waters C18 column (250 mm × 4.6 mm, 5 µm) was used for separation at 254 nm wavelength and 25 °C column temperature. The flow rate was set at 0.7 ml/min with injection volume of 10 µl. The mobile phase was consisted of solvent A (Methanol) and solvent B (Water + 0.5% phosphoric acid) at a ratio of 50:50 v/v for 7 minutes. The signals were acquired and processed in a computer (HP) using the software ChemStation.

Rice cultivation and snails rearing: The rice was cultivated continuously in the glasshouse at Field 2, Universiti Putra Malaysia to feed the snails and to be used in bioassay experiments. Rice variety MR 219 was directly seeded into plastic containers (28 cm × 39 cm × 11 cm) filled with clay soil. The snails were collected from Tanjung Karang paddy field, Selangor, Malaysia (3°25′27″ N 101°11′05″ E). The eggs, juveniles (hatchlings) and adults were handpicked. The egg masses were placed in a separate tank as they had to be kept away from water. The snails were reared in a plastic aquarium (15 cm × 41 cm × 20 cm) in the glasshouse under natural condition. Throughout the study, the aquariums were washed and the water was changed every two days to avoid contamination. Adult snails were provided with rice leaves of up to 28 days old for their consumption, whereas, one to 20 days old hatchlings were fed with algae. The black apple snails were identified based on their shell morphology (Cowie *et al*., 2006). Snails with shell height of 4 cm were used in all experiments.

Mortality bioassay: Mortality bioassay was carried out based on the guidelines for molluscicide evaluation (WHO, 1983) with slight modifications. The crude extracts of fruits and leaves were tested on *P. maculata* at five different concentrations (1000, 750, 500, 250 and 100 ppm) along with positive control synthetic molluscicide Niclosamide (1.12 ml in 200 ml of water) and negative control distilled water. For each treatment, 200 ml of the respective solution was added to a plastic aquarium containing five apple snails. The snails were starved for 24 hours prior to the experiment, and 0.5 g of rice leaves were provided to the snails for feeding during the experiment. The experiment was done under Completely Randomized Design with five replications. The mortality was assessed at 24, 48 and 72 hours. If the apple snails failed to show coordinated movements when softly pushed were considered dead.

Feeding deterrent bioassay: Feeding deterrent bioassay was conducted through leaf dip bioassay method described by Dawidar *et al*. (2012) with slight modifications. Five concentrations (1000, 750, 500, 250 and 100 ppm) of both crude extracts were tested on black apple snails. Niclosamide and water were used as positive and negative controls, respectively. Five apple snails per aquarium were used in all five replications. Apple snails were starved for 24 hours before the experiment. The leaf area and weight of rice leaves were measured using LI-3100 Leaf Area Meter (LI-COR, USA) and Sartorius BT224S analytical balance (Sartorius, Germany) before and after exposure.

Statistical analysis: The experiments were carried out in CRD, and data were analysed by ANOVA for LSD test at 0.05 probability level using SAS 9.4 computer software (SAS Institute Inc. 2009). The data obtained from mortality bioassay were normalized using arcsine transformation.

RESULTS

Extraction and quantification of saponin bioactive compound: The crude extract of fruits, leaves and stem-barks of *P. macrocarpa* were used for saponin quantification. Before HPLC analysis of samples, the external standard system was used to optimize the method as it was necessary for the quantification of the compound in samples (Mradu *et al*., 2012). The standard saponin from Sigma-Aldrich at five different concentrations (30, 25, 20, 15, and 10 ppm) were used to establish and calibrate the HPLC method at R^2 (0.999) for saponin quantification. The retention time for saponin detection was recoded from 2.12 to 2.17 minutes. Table 1 shows the retention time, peak area, peak height, saponin content in 30 ppm concentration of sample and saponin yield percentage obtained from the crude extracts of each plant parts that were quantified using HPLC analysis.

The highest saponin contents were 24.7 ppm with the yield percentage of 14.8 %. Saponin was detected in the fruit extracts at the retention time of 2.134 minutes with peak area of 150 μV^*sec and peak height 7.46 µV. The second highest saponin contents were 22.7 ppm with the yield percentage of 14.4 % and that were detected at the retention time of 2.112 minutes with peak area of 139.7 μV^*sec and peak height 5.72 µV in the leaf extract. Meanwhile, the crude extracts of stem barks recorded the least amount of saponin which was 5.9 ppm with yield percentage of 2.4 %. Saponin in stem barks of *P. macrocarpa* was detected at the retention time of 2.108 minutes with peak area of 53.48 μV^*sec and peak height 3.11 µV.

Mortality bioassay: Table 2 shows the mortality percentage of *P. maculata* in different concentrations of fruits and leaves extracts of *P. macrocarpa at* 24, 48 and 72 hours. At 24 hours, both plants parts showed positive molluscicidal activities against *P. maculata at* 500, 750 and 1000 ppm concentrations. The highest mortality percentage recorded at 24 hours was 44% in 1000 ppm of leaves extract followed by 36% in 750 ppm of same crude extract and 28% in 1000 ppm of fruits extract as compared to 100% mortality in positive control niclosamide.

All treatments were significantly different from positive control niclosamide at $P<0.05$. The lowest mortality percentage recorded was 8% in 750 ppm of fruits extracts. Meanwhile, no dead snails were found at 24 hours in 100 and 250 ppm of both crude extracts and negative control water. After 48 hours, the highest mortality was 100% recorded at 1000 ppm and 750 ppm of both crude extracts followed by 56% and 52% in 500 ppm of leaves and fruits extracts respectively. There were no dead snails found in 250 and 100 ppm of leaves and fruits extracts, as well as in untreated control at 48 hours. Leaves and fruits extract applied in higher concentrations at 1000 pm and 750 ppm were not significantly different from positive control niclosamide at 48 hours. This meant that the molluscicidal effects of both plants' parts were similar to niclosamide. After exposure of 72 hours, the mortality percentage in 500 ppm of leaves and fruits crude extracts reached 80% and 68% respectively. However, no mortality was recorded in 250 ppm and 100 ppm of both crude extracts and water until 72 hours.

Feeding deterrent bioassay: Table 3 illustrates the mean weight of rice leaves consumed by *P. maculata* when exposed to the crude extracts of leaves and fruits. After 24 hours, the mean leaf weight consumed by black apple snails for positive control treated with niclosamide was 0.00 ± 0.00 g. Similarly, leaves showed no reduction in weight when treated with 1000 ppm concentration of leaves extract. The lower the consumed leaf weight, the better the effects of crude extracts in disrupting the feeding behaviour of *P. maculata.*

The consumed leaf weight for extracts from both fruits and leaves at 1000, 750 and 500 ppm concentrations were not significantly different from niclosamide at $P>0.05$, therefore, it showed similar antifeedant effects as niclosamide. The lowest consumed weight of leaves was 0.00 ± 0.00 g in 1000 ppm concentration of leaves extracts followed by 0.002 ± 0.002 g at 1000 ppm concentration of fruits extract. Meanwhile, the 250 and 100 ppm concentrations of leaves and fruits extracts were significantly different from control niclosamide at $P<0.05$. The highest mean consumed weight recorded was 0.096 ± 0.006 and 0.096 ± 0.009 g at 100 ppm concentrations of leaves and fruits extracts respectively. The consumed weight of leaves at 250 and 100 ppm of both crude extracts were also significantly different at $P<0.05$ from negative control water which was 0.37 ± 0.008 g. Table 4 shows the leaf area consumed by *P. maculata* when exposed to 1000, 750, 500, 250 and 100 ppm concentrations of fruits and leaves extracts. After 24 hours, the lowest mean leaf area was 0.00 ± 0.00 cm^2 when treated with positive control niclosamide. Lower leaf area means, reflected better effects of crude extracts in disrupting the feeding behaviour of *P. maculata.*

In terms of leaf area consumption, the three highest concentrations of both crude extracts were not significantly different with the control niclosamide at $P>0.05$. This proved that 1000, 750 and 500 ppm concentrations of both crude extracts have the same antifeedant effects as niclosamide. However, the lowest mean area of leaves for crude extracts recorded was 0.02 ± 0.02 cm^2 at 1000 ppm concentration of leaves extracts followed by 0.122 ± 0.122 cm^2 at 1000 ppm concentration of fruit extract respectively. The 250 ppm and 100 ppm concentrations of both crude extracts showed significant difference with negative control water at $P<0.05$. The mean highest consumed leaf area recorded was 6.474 ± 0.24 cm^2 followed by 5.538 ± 0.56 for 100 ppm concentrations of leaves and fruits extracts, respectively.

Table 1: Saponin contents (ppm) in 30 ppm concentration and saponin yield (%) from different parts of *P. macrocarpa.*

Plant Parts	Ret. Time (min)	Area (μV*sec)	Height (μV)	Area %	Saponin contents (ppm)	Saponin yield %
Fruits	2.134	150.01	7.46	100	24.7	14.8
Leaves	2.112	139.73	5.72	100	22.7	14.4
Stem-barks	2.108	53.48	3.11	100	5.9	2.4

Table 2: Mortality Percentage (%) of *P. maculata* when treated with crude extracts of leaves and fruits at 24, 48, and 72 hours.

Treatments	Concentration (ppm)	Mean±SE		
		24h	48h	72h
Fruit Extract	1000	28 ±4.89cd	100 ±0.0a	100 ±0.0a
	750	8 ±8.0ef	100 ±0.0a	100 ±0.0a
	500	12 ±4.89ef	52 ±10.19b	68 ±4.8c
	250	0±0.0f	0±0.0c	0±0.0d
	100	0±0.0f	0±0.0c	0±0.0d
Leaves Extract	1000	44 ±7.48b	100 ±0.0a	100 ±0.0a
	750	36 ±7.4bc	100 ±0.0a	100 ±0.0a
	500	20 ±6.32de	56 ±7.4b	80 ±8.9b
	250	0±0.0f	0±0.0c	0±0.0d
	100	0±0.0f	0±0.0c	0±0.0d
Control	Niclosamide	100 ±0.0a	100 ±0.0a	100 ±0.0a
	Untreated	0±0.0f	0±0.0c	0±0.0d

Means with same letters within column are not significantly different at P > 0.05

Table 3: Mean leaf weight (g) consumed by *P. maculata when* treated with crude extracts of leaves and fruits.

Treatments	Concentration (ppm)	Mean±SE
		Weight
Fruit Extract	1000	0.002 ±0.002e
	750	0.004 ±0.002e
	500	0.01 ±0.003e
	250	0.072 ±0.015c
	100	0.096 ±0.006b
Leaves Extract	1000	0.00 ±0.00e
	750	0.006 ±0.004e
	500	0.012 ±0.005e
	250	0.05 ±0.004d
	100	0.096 ±0.009b
Control	Niclosamide	0.00 ±0.00e
	Untreated	0.37 ±0.008a

Means with same letters within column are not significantly different at P > 0.05

Table 4: Mean leaf area (cm^2) consumed by *P. maculata* when treated with crude extracts of leaves and fruits.

Treatments	Concentration (ppm)	Mean±SE
		Area
Fruit Extract	1000	0.122 ±0.122e
	750	0.404 ±0.24e
	500	0.714 ±0.28e
	250	3.902 ±0.33c
	100	6.474 ±0.24b
Leaves Extract	1000	0.02 ±0.02e
	750	0.482 ±0.288e
	500	0.884 ±0.38e
	250	2.306 ±0.58d
	100	5.538 ±0.56b
Control	Niclosamide	0.00 ±0.00e
	Untreated	15.982 ±0.48a

Means with same letters within column are not significantly different at P > 0.05

DISCUSSION

Extraction and quantification of saponin bioactive compound: Previous study on the crude extract from the fruits of *P. macrocarpa* showed the presence of bioactive compounds such as saponin glycosides, tannins, phenols, and flavonoids (Lay *et al*. 2014). Altaf *et al*. (2013) and Andrean *et al*. (2014) had also highlighted that the fruits of *P. macrocarpa* are rich in saponin bioactive compound. Similarly, the HPLC analysis results of this study supported this observation. The fruit extracts of *P. macrocarpa* contained 82.4% of saponin while the yield of crude extract from dried powder of fruits of *P. macrocarpa* was 18 % so the total yield of saponin from fruits was recorded 14.8 %. This strongly supports the *P. macrocarpa* fruits as a main source of saponin bioactive compound, thus, confirming that saponin was the main bioactive compound in the fruits of *P. macrocarpa.* This finding is crucial, as future extraction of saponin from this plant can be targeted on the fruits as it can yield high quantity.

As detected by the HPLC analysis, the leaves extracts were found to be the second richest in saponin content. Total saponin amount was 75.7 % while the yield of crude extract from dried powder of Leaves of *P, macrocarpa* was 19% therefore; the saponin yield % was 14.4 in leaves. Previous studies have reported the presence of saponin bioactive compound in the leaves of *P. macrocarpa,* together with tannins and flavonoids (Shodikin, 2010; Faried *et al*., 2016). Previously, some other studies also reported presence of saponin and alkaloids in leaf extracts of *P. macrocarpa* and proposed the use of *P. macrocarpa* leaves for antibacterial purposes (Elianora *et al*., 2017; Altaf *et al*., 2013). Furthermore, the yield of crude extract from leaves was higher comparing with fruits. This further proved that the leaves are also a suitable source of saponin.

The HPLC analysis of the stem-barks extracts revealed the lowest saponin content in comparison to all the other plant parts. Although previous studies have reported the presence of saponin bioactive compound in the stem-barks of *P. macrocarpa* (Gotama *et al*., 1999; Altaf *et al*., 2013). But based on the HPLC analysis in this study, only 5.94 ppm of saponin was detected in 30 ppm of crude extract which were only 19.8 % and the yield of crude extracts was also low as it was only 12% and total saponin yield from stem-barks was only 2.4%. The low saponin content in the barks reports as an inefficient source of saponin as compared to the leaves and fruits of *P. macrocarpa.*

Mortality bioassay: The results obtained from mortality bioassay proved that saponin extracted from fruits and leaves of *P. macrocarpa* have the potential to kill apple snails as early as 24 hours. Previous studies had also recorded the molluscicidal effects of saponin from *Furcraea* spp. on apple snails at 24 hours (Osman *et al*., 2011; Jose *et al*., 2013; Mokhtar, 2016). It was also observed that saponin from fruits and leaves could show 100% mortality after 48 hours. Similarly, some previous studies also revealed that saponin from *Entada phaseolides* could achieve 80 to 100% mortality against apple snails after 48 hours of exposure (Morallo-Rejesus and Maini, 1991; Morallo-Rejesus *et al*., 1995; Rejesus and Punzalan, 1997). As observed in this study, the effectiveness of both crude extracts was directly relative to the time of exposure to the saponin concentration. If the exposure time to saponin increased the egg laying capacity, growth rate and survival rate of freshwater apple snails also decreased (Mahato *et al*., 1982).

Feeding deterrent bioassay: The results of this study from feeding deterrent bioassay revealed that apple snails avoid the saponin treated diet, some previous studies also recorded 0% leaf damaged in lettuce caused by snails at 4 hours when treated with 3% ethanolic Myrrh (Ali, 2005). Another study also reported 19% damage in rice seedlings when treated with methanolic extract of dried neem leaves (Latip *et al*., 2017). Higher saponin contents lowered the feeding activities of snails, as saponin displayed molluscicidal properties against apple snails (Huang *et al*., 2003). Additionally, the noxious odour produced can also prevent apple snails from consuming rice leaves. Higher concentration of crude extracts of saponin containing plants, reduced the feeding attraction of apple snails towards rice leaves (Mokhtar, 2016). Besides of saponin's direct molluscicidal and insecticidal activities, it is also very well-known for its antifeedant properties. Therefore, plants that containing saponin could also serve as an antifeedant to prevent molluscs from feeding on living plants. (Mason *et al*., 1994; Chaieb *et al*., 2009), This supported the finding in the anti-feedant bioassays conducted in this research.

Conclusion: Methanolic extracts of fruits and leaves of *P. macrocarpa* are rich in saponin and showed strong bioactivities against black apple snails. Therefore, it is recommended that it should be further investigated as an eco-friendly cost effective botanical molluscicide.

REFERENCES

Ali, A., (2005). Repellent, Antifeedant & Molluscicidal Effects of *Commiphora* spp. Oleoresins, and their extracts, on *Deroceras reticulatum* and *Helix aspersa* (Doctoral thesis). Cardiff University, United Kingdom.

Altaf, R., M. Z. B. Asmawi, A. Dewa, A. Sadikun and M. Umar (2013). Phytochemistry and medicinal properties of *Phaleria macrocarpa* (Scheff.) Boerl. extracts. Pharmacogn. Rev. 7: 73. https://doi.org/10.4103/0973-7847.112853

Andrean, D., S. Prasetyo, A.P. Kristijarti and T. Hudaya (2014). The Extraction and Activity Test of Bioactive Compounds in *Phaleria Macrocarpa* as Antioxidants. Procedia Chem. 9: 94–101. https://doi.org/10.1016/j.proche.2014.05.012

Arfan, A.G., R. Muhamad, D. Omar, A.A.N. Azwady and G. Manjeri (2014). Distribution of two *Pomacea* spp . in Rice Fields of Peninsular Malaysia. Annu. Res. Rev. Biol. 4: 4123–4136. https://doi.org/10.9734/ARRB/2014/11398

Chaieb, I., M. Kamel, H. Hialima and M.H. Hamouda (2009). Antifeedant activity of *Cestrum parqui* crude saponic extract . Tunis. J. Med. Plants Nat. Prod. 1: 27–33.

Cowie, R.H., (2005). The Golden Apple Snail : *Pomacea* species including Pomacea canaliculata (Lamarck , 1822) (Gastropoda : Ampullariidae).

Cowie, R.H., (1998). Patterns of introduction of non-indigenous non-marine snails and slugs in the Hawaiian Island. Biodivers. Conserv. 7: 349–368.

Cowie, R.H., K.A. Hayes and S.C. Thiengo (2006). What are the apple snails? Confused txanomy and some preliminary resolution, in: Joshi, R.C., Sebastian, L.S. (Eds.), Global Advances in Ecology and Management of Apple Snails. pp. 2–23.

Dawidar, A.E.M., M.M. Mortada, H.M. Raghib and M. Abdel-Mogib, (2012). Molluscicidal activity of *Balanites aegyptiaca* against Monacha cartusiana. Pharm. Biol. 50: 1326–1329. https://doi.org/10.3109/13880209.2012.674950

De Geyter, E., E. Lambert, D. Geelen and G. Smagghe (2007). Novel advances with plant saponins as natural insecticides to control pest insects. Pest Technol. 1: 96–105. https://doi.org/ 10.1.1. 455.4613

Eldredge, L.G., (1994). Perspectives in aquatic exotic species management in the Pacific Islands. Pacific Sci. Assoc., Honolulu, hawaii, USA

Elianora, D., B. Busman and Y. Amrilya (2017). Activities test of Mahkota Dewa (*Phaleria macrocarpa*) leaves extract against Candida albicans of HIV/AIDS patients. Padjadjaran J. Dent. 29: 1–7. https://doi.org/10.24198/ pjd.vol29no1.11588

Faried, A., H. Bolly, L. Septiani, D. Kurnia, M. Arifin and F. Wirakusumah (2016). Potential of Indonesian Herbal Medicine, *Phaleria macrocarpa* (Scheff.) Boerl, for Targeting Multiple Malignancy Signaling Pathways: An Introductory Overview. European J. Med. Plants. 11: 1–17. https://doi.org/10.9734/ EJMP/2016/20760

Gotama, I.B.I., S. Sugiarto, M. Nurhadi, Y. Widiyastuti, S. Wahyono and I.J. Prapti (1999). Inventaris tanaman obat Indonesia. 5th Ed. Deptt. kesehatan Bandan. Pen. dan Peng. Jakarta.

Guo, S.,J. Duan, Y. Tang, Y. Qian, J. Zhao, D. Qian, S. Su and E. Shang, (2011). Simultaneous qualitative and quantitative analysis of triterpenic acids, saponins and flavonoids in the leaves of two Ziziphus species by HPLC-PDA-MS/ELSD. J. Pharm. Biomed. Anal. 56: 264–270. https://doi.org/10.1016/j.jpba.2011.05.025

Hayes, K.A., R.H. Cowie, S.C. Thiengo, and E.E Strong (2012). Comparing apples with apples: Clarifying the identities of two highly invasive Neotropical Ampullariidae (Caenogastropoda). Zool. J. Linn. Soc. 166: 723–753. https://doi.org/10.1111/j.1096-3642.2012.00867.

Hending, W., (2009). Benzophenone glucoside isolated from the ethyl acetate extract of the bark of mahkota dewa (Phaleria macrocarpa (Scheff.)

Boerl.) and its inhibitory activity on leukemia L1210. Indo J. Chem. 9 (1):142-145.

Hostettmann, K., H. Kizu and T. Tomimori (1982). Molluscicidal properties of various saponins. Planta Med. 44: 34–35. https://doi.org/10.1055/s-2007-971396

Huang, H.C., S.C. Liao, F.R. Chang, Y.H. Kuo and Y.C. Wu (2003). Molluscicidal saponins from Sapindus mukorossi, inhibitory agents of golden apple snails, *Pomacea canaliculata*. J. Agric. Food Chem. 51: 4916–4919. https://doi.org/10.1021/jf0301910

Jose, I., A. Lorena and A. Hildebrando (2013). Toxicity of the Biopesticides *Agave americana*, *Furcraea andiana* (Asparagaceae) and *Sapindus saponaria* (Sapindaceae) on invader snail *Melanoides tuberculata* (Thiaridae) Neotrop. Helminthol. 7: 2218–6425.

Kim, W.-J., B. Veriansyah, Y.W. Lee, J. Kim and J.D. Kim (2010). Extraction of mangiferin from Mahkota Dewa (*Phaleria macrocarpa*) using subcritical water. J. Ind. Eng. Chem. 16: 425–430. https://doi.org/10.1016/j.jiec.2009.08.008

Latip, S., H.M. Noor, M.F. Keni and R. Rosli (2017). Antifeedant Activities of Neem Seed Extracts for Controlling Golden Appple Snail, *Pomacea canaliculata*. Soc. Sci. 12: 294–298.

Lay, M.M., S.A. Karsani, B. Banisalam, S. Mohajer and S.N. Abd-Malek (2014) Antioxidants, phytochemicals, and cytotoxicity studies on *Phaleria macrocarpa* (Scheff.) Boerl Seeds. Biomed Res. Int. 1-13. https://doi.org/10.1155/2014/410184

Lowe, S., M. Browne, S. Boudjelas and M. De-Poorter (2000). 100 of the world's worst invasive alien species a selection from the global invasive species data base. The Invasive Species Specialist Group (ISSG)., Aliens.

Mahato, S.B.,A.N. Ganguly and N.P. Sahu (1982). Steroid saponins. Phytochemistry 21: 959–978. https://doi.org/10.1016/S0031-9422(00)82400-0

Mason, W.M.A., G.S. Puritch and D.S. Almond (1994). Saponin containing anti-feedant and molluscicide for terrestrial mollusk control. Patent No. US 5290557 A.

Mokhtar, A, S., (2016). Nano emulsion formulation of saponin to control black apple snails, *Pomacea maculata* Perry (Doctoral thesis). Univ. Putra Malaysia, Serdang, Malaysia.

Morallo-Rejesus, B and P.N. Maini (1991). Sapogenic plant material for golden snail (Pomacea spp.) control. Phase-I.

Morallo-Rejesus, B., P.N. Maini and E. Punzalan (1995). Sapogenic plant materials foe golden snail (Pomacea spp.) control. Phase-II.

Mradu, G., S. Saumyakanti, M. Sohini, and M. Arup (2012). HPLC profiles of standard phenolic compounds present in medicinal plants. Int. J. Pharmacogn. Phytochem. Res. 4: 162–167.

Mustarichie, R., Z. Udin, A. Muhtadi, E. Surahman and A. Subarnas (2012). The antioxidant activity and cytotoxicity of methanol extracts from cranberry plants. Int. Res. J. Pharm. Appl. Sci. 2: 56–61.

Naylor, R., (1996). Invasions in Agriculture: Assessing of the Golden Apple Snail in Asia the. Ambio 25: 443–448.

Osman, G.Y., A.M. Mohamed, A.A Kader and A.A. Mohamed (2011). Biological studies on Biomphalaria alexandrina snails treated with *Furcraea selloa* marginata plant (family: Agavaceae) and *Bacillus thuringiensis* kurstaki (Dipel-2x). J. Appl. Pharm. Sci. 1: 47–55.

Rawlings, T.A., K.A. Hayes, R.H. Cowie, and T.M. Collins (2007). The identity, distribution, and impacts of non-native apple snails in the continental United States. BMC Evol. Biol. 7: 1–14. https://doi.org/10.1186/1471-2148-7-97

Rejesus, H.M. and E.G. Punzalan (1997). Molluscicidal Action of Some Philippine Plants on Golden Snails , Pomacea Spp . Phil. Entomol. 11: 65–79.

San Martin, R. (2007). Recent development in the use botanical molluscicides against apple snail, (*Pomacea canaliculata*)., in: R.C., J., L.S., S. (Eds.), Global Advances in Ecology and Management of Golden Apple Snails. PhilRice, Neur Ecija, Philippines, pp. 393–403.

Shodikin, M.A. (2010). Antimicrobial activity of mahkota dewa (*Phaleria macrocarpa* (Scheff). Boerl) leaf extract againts *Pseudomonas aeruginosa* by agar dilution and scanning electron microscopy. Folia Medica Indones. 46, 172–178.

Sparg, S.G., M.E. Light and J. Van Staden (2004). Biological activities and distribution of plant saponins. J. Ethnopharmacol. 94: 219–243. https://doi.org/10.1016/j.jep.2004.05.016

Takeuchi, T.M., C.G. Pereira, M.E.M. Braga, M.R. Maróstica, P.F. Leal and M.A.A. Meireles (2009). Low-pressure solvent extraction (solid–liquid extraction, microwave assisted, and ultrasound assisted) from condimentary plants., in: Meireles, M.A.A. (Eds. . (Ed.), Extracting Bioactive Compounds for Food p Roducts—Theory and Applications. CRC Press, Boca Raton, pp. 151–158.

Tjandrawinata, L., D. Nofiarny, L.W. Susanto, P. Hendri and A. Clarissa (2011). Symptomatic treatment of premenstrual syndrome and/or primary dysmenorrhea with DLBS1442, a bioactive extract of *Phaleria macrocarpa*. Int. J. Gen. Med. 465. https://doi.org/10.2147/IJGM.S21053

WHO, (1983). Reports of the scientific working group on plant molluscicides. Bull. World Heal. Organ. 61,

927–929.

https://doi.org/http://apps.who.int/iris/handle/10665/60086

Yahaya, H., M. Nordin, M.N. Mohamad-Hisham and A. Sivapragasam (2007). Golden apple snails in Malaysia., in: Joshi, R.C., Sebastian, L.S. (Eds.), Global Advances in Ecology and Management of Golden Apple Snails. pp. 215–230.